Lateral organization of cholesterol molecules in lipid-cholesterol assemblies

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We present results of an off-lattice simulation of a two-component planar system, as a model for lateral organization of cholesterol molecules in lipid-cholesterol assemblies. We explore the existence of "superlattice" structures even in fluid systems, in the absence of an underlying translational long-range order, and study their coupling to hexatic or bond-orientational order. We discuss our results in context of geometric superlattice theories and "condensation complexes" in understanding a variety of experiments in artificial lipid-cholesterol assemblies.

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I. INTRODUCTION

In real biological membranes as well as in artificial lipid assemblies with many components, the cholesterol molecules are often dispersed in large concentrations in a sea of various kinds of lipids. These molecules can strongly influence the physical properties of the membrane, such as its fluidity, rigidity, and the thermodynamics of gel to fluid transition. They perhaps even play a crucial role in biological cell-signaling processes through formation of transient nanoscale domains, called rafts [1]. The lateral organization of the cholesterol molecules in the lipid layers is central to these kinds of behavior and remains a very active area of research.

The thermotropic phase transition in lipids and lipid-cholesterol assemblies have been studied extensively [2–6]. In the absence of cholesterol, a pure lipid membrane shows a sharp "gel" to fluid thermotropic phase transition. At high temperatures, the membrane is fluid with high lateral mobility of the molecules in the plane, and disorder in the conformational states of the acyl chains. As the temperature is lowered, the membrane undergoes a sharp transition to a low temperature gel state characterized by (quasi-long-range translational order resulting from the freezing of the lateral motion of the molecules and a high degree of conformational order among the acyl chains. Clearly, the transition consists of two parts corresponding to the freezing of lateral motion and chain ordering—for the pure lipid system, these two are thermodynamically coupled, occurring at the same temperature. The incorporation of cholesterol decouples these two transitions leading to the formation of a new "liquid-ordered" state [2]—where the acyl chains have ordered, but there exists lateral mobility of the molecules. This raises the question: How homogeneous is the distribution of cholesterol molecules within the membrane?

There have been several experimental studies, where the cholesterol concentration is carefully controlled and its effects on the phase diagram and the thermodynamic properties are assessed [7]. A growing number of these studies have been interpreted in terms of a "superlattice" of cholesterol molecules in the lipid layers [7–10]. In particular, the "magic concentrations" seen in these experiments have a very simple, purely geometrical interpretation of cholesterol molecules arranging themselves within a triangular-lattice-like arrangement of amphiphilic molecules, in such a way that there are no nearest-neighbor cholesterols ($f \approx 33\%$), there are no nearest and second-neighbor cholesterols ($f \approx 15\%$), etc. Such regular distribution has been observed in several different systems. These include cholesterol assemblies with various kinds of phospholipids and sphingolipids, other lipid-sterol mixtures (e.g., dehydroergesterol and phospholipids [11]), phospholipid-cardiolipin [12] and Pyrenyl-acyl fatty acid (PyrFA) systems [13], and even binary mixtures of two different types of lipids (e.g., PE-PC systems [14]). Thus, while we discuss our results in the context of lipid-cholesterol systems, they are, in fact, applicable to a wide variety of experimental systems.

It is well known that the lipid system is fluid under biological conditions, and does not, under any conditions, form a true long-range ordered triangular lattice. The local coordination of the lipid molecules at the level of headgroups or at the level of acyl chains resembles a triangular lattice, as expected for any two-dimensional system of closed-packed hard disks or spheres. It is also known that although two-dimensional systems do not have translational long-range order, they can develop a true hexatic or bond-orientational order at low temperatures. Thus an interesting question from a statistical mechanics point of view is: Can lateral superlattice arrangement of cholesterol molecules arise in the absence of translational order and how is it influenced by the onset of bond-orientational or hexatic order in the system? This is the issue that we plan to address here.

Previous numerical studies of the gel to fluid transition in lipid-cholesterol assemblies and the formation of superlattices have used a triangular lattice description of the membrane [15–22]. While these studies were able to capture several salient features of the thermotropic transition and even the formation of superlattices, such models cannot, obviously, capture the effects of lateral mobility of the molecules. Using an off-lattice description, Nielsen and co-workers [23–25] showed that the two microscopic transitions can be decoupled by simply varying the parameters of the Hamiltonian. They have addressed in detail the issue of molecular organizations, including lateral distribution of cholesterol, in the vicinity of the chain-melting transition and in the "liquid ordered" state.

In this paper, we use a Metropolis Monte Carlo algorithm to develop an off-lattice simulation of a membrane in the vicinity of the "head group ordering" transition, focusing primarily on the lateral distribution of the cholesterol molecules. The model consists of two types of molecules, both of whom have a hard core repulsion and one of them have additional longer-ranged repulsive interaction. The molecules with just a hard-core represent lipids and those with additional longer-ranged interactions represent cholesterols. The molecules are free to move in a plane, constrained only by their mutual interactions.

The effective longer-range repulsive interactions between the cholesterol molecules arise from both potential energies of interaction and steric mismatch between the sizes of the lipid headgroups ($\sim$65 Å^2 for phospholipids and $\sim$35 Å^2 for cholesterol). The origin and nature of the steric interaction between cholesterol molecules have been discussed in detail by Somerharju *et al.* [10]. In addition to the mismatch in the sizes of their headgroups, the cholesterol molecules are much more rigid than the lipid chains. The flexibility of the chains allows them to pack well together, unlike the hard cholesterols which cannot pack well [26]. The tails can also pack well around the cholesterol molecules. A closer packing leads to a larger van der Waals attraction between the molecules. This lack of potential energy gain further keeps the cholesterols from being next to each other, and acts as an effective repulsion. An even longer-ranged interaction arises from the finite dipole moment of the cholesterol molecules perpendicular to the plane of the bilayer (the in-plane dipole moment averages to zero). This dipolar interaction is repulsive, rather than attractive, in nature since the amphiphilic cholesterol molecules are forced to align parallel to each other with the hydrophilic (hydrophobic) end pointing towards the surface (center) of the bilayer.

In this paper, we are primarily interested in how the density (or pressure) and temperature affect the phase-behavior and lateral organization of the cholesterol molecules in the layers. Our key finding is that a superlattice arrangement, where every cholesterol molecule is completely surrounded by lipids, can arise even in the absence of translational or hexatic order. The exclusion of cholesterol-cholesterol neighbors is a local issue that can happen gradually as the temperature is lowered. This gradual crossover may or may not couple to the bond-orientational order transition in these systems. Depending on the particle density, the transition can become cooperative and turn into a sharp phase transition. It is also important to note that our results suggest that the superlattice formation is driven by a generic minimal two-body longer-ranged repulsion between the cholesterol molecules and does not depend on the details of the interaction between the different membrane constituents.

These results lend strong support to the idea of "magic fractions" and superlattices in lipid-cholesterol assemblies. Just an effective repulsion between cholesterols is enough to cause their lateral organization, which can become rather complete as the temperature is lowered. A longer-range ordered lattice is not necessary for this to happen, and it can happen even while the assembly is in the fluid phase. One can contrast these ideas with those of "condensation complexes" [27,28], where lipids and cholesterols bond together to form giant, almost molecular complexes. And these complexes are then argued to be behind the anomalous thermodynamic behavior. In both pictures, the lateral organization of the cholesterol molecules result. The key difference is that, in the former, there is no clear bonding between cholesterols and lipids nor a distinction between intracomplex and intercomplex neighbors, which appears artificial to us in a close-packed system. In this sense, the superlattice picture is much more appealing.

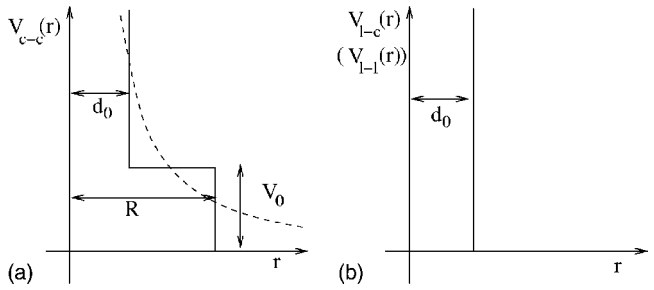

FIG. 1. The potentials for the interactions between different membrane constituents. (a) Effective cholesterol-cholesterol interaction; (b) the lipid-cholesterol and lipid-lipid interactions are assumed to consist of just a hard-core repulsion. For simplicity, the hard-core radius for both the cholesterol and lipid molecules are assumed to be the same. All distances are measured in units of a. Potential energies are in arbitrary units.

The plan of the paper is as follows. In Sec. II, we discuss our model and simulation techniques. In Sec. III, the results for a one-component hard-disk system are discussed. In Sec. IV, we present our simulation results for the two-component system. In Sec. V, we discuss the implications of our results and present our conclusions and future research directions.

II. MODEL AND SIMULATION TECHNIQUE

We denote the cholesterol-cholesterol, cholesterol-lipid, and lipid-lipid interactions by V_{c-c}, V_{c-l}, and V_{l-l}, respectively, and assume that they only depend on the distance between the molecules. The dependence of the interaction potentials on distance are shown in Fig. 1. The lipids interact with each other and with the cholesterols only via a hard-core repulsion. The cholesterol molecules, in addition to the hard-core repulsion ($r<d_0$), experience a longer-range repulsive interaction (V_0) with each other ($d_0<r<R$). The magnitude of the repulsive interaction V_0 defines the energy scale for the problem.

The molecules are confined to move in a system of fixed size (fixed volume ensemble). We choose a region that is a parallelogram, where all sides have length L and the acute angle is 60°. With the total particle number N we define $a^2=L^2/N$. Thus the volume fraction of the molecules for a given value of the hard-core distance d_0 is $\pi d_0^2/2\sqrt{3}a^2$. The value of R is chosen to be $R=1.3a$. The set of dimensionless parameters we work with are $T/V_0, d_0/a$, and R/a. We have studied only one cholesterol concentration, where the ratio of the number of lipid to cholesterol molecules is fixed at 2:1.

A Monte Carlo (MC) method is used to simulate the system. We start our simulations with the particles placed at the

sites of a triangular lattice. Each MC step consists of two types of updates: (i) each particle is moved by a small amount and the move is accepted or rejected by Metropolis algorithm, and, (ii) a pair of nearest neighbor lipid cholesterol is "swapped." Once again a Metropolis scheme is used to accept or reject the move.

We start from a high temperature, where the thermal energy is larger than the characteristic energy scale set by the cholesterol-cholesterol interaction strength. The system is allowed to thermalize for 2×10^5 MC steps followed by another 5×10^5 MC steps during which measurements are taken. Next the temperature is lowered in small decrements and the whole procedure of thermalization and measurements is repeated for each temperature. Typically such a MC code would sample over the entire system at each step—thus making the algorithm very slow. However, since the range of interactions is finite, we can divide the system into several regions (typically four times the size of the unit cell of the starting configurations). The particles in each region will interact only with other particles in the same region and neighboring ones. This leads to a significant increase in the efficiency of the code. As a test of the MC code, we always start the simulations with an initial configuration where the cholesterol molecules are all placed at one end of the system. Such a state has a very high energy and should quickly settle down to a more even distribution. We have also performed additional checks by starting fresh runs at each temperatures and comparing the results with those obtained from gradual quenching from higher temperatures. The results agree, but we find that the equilibration time increases rapidly with decreasing temperature and it is more efficient to use the quenching method we have adopted here.

III. RESULTS

A. Hard-disk system

One of the advantages of the above model is that if the longer-range effective cholesterol-cholesterol interaction is ignored, the model reduces to a system of hard disks in two dimensions. Such a system is well studied and is known to undergo a two-dimensional (2D) melting transition as the density of particles is varied [29]. For large density (characterized by large hard core radius), the system has long range bond orientational order, whereas at low densities the system is a uniform fluid. The order parameter for the transition was defined by Halperin and Nelson and Young [30] as

$$\psi(i) = \frac{1}{z(i)} \sum \exp(-6\ \theta_{ij}),$$

where $z(i)$ is the number of nearest neighbors of the particle i (defined as $r_{ij} < r_{nn}$) and θ_{ij} is the angle made by particles i and j with some reference axis. The sum runs over the nearest neighbors of the particle i. The ordered phase is characterized by a nonzero value of $\langle \psi(i) \rangle$, averaged over the entire system. The results for our simulation of the hard core system is shown in Fig. 2. As the hard core radius is decreased, the system goes through a phase transition from an ordered to a fluid state. There is debate in the literature about the nature of the transition—whether it is continuous or discontinuous [29]. We will not attempt to throw further light on this difficult theoretical issue, rather, we will make use of the estimate of critical hard-core radius to study the two-component system on both sides of the transition.

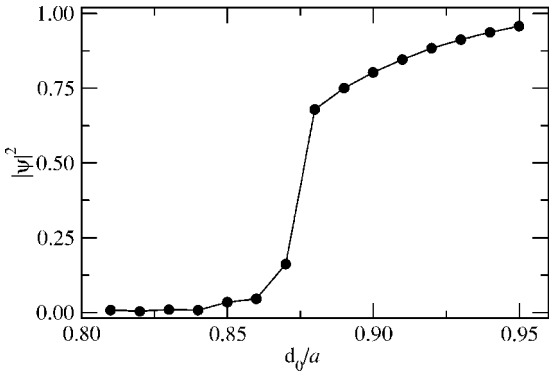

FIG. 2. The bond orientational order parameter as a function of varying hard-core radius for a system of hard disks.

B. Lipid-cholesterol systems

Next we turn on the longer-range interaction between the cholesterol molecules and investigate the development of cholesterol ordering (if any) as a function of temperature. At high temperatures, when the thermal excitation energy is of the order of the cholesterol-cholesterol interaction strength V_0, the lipid and cholesterol molecules are completely disordered. As the temperature is lowered, the cholesterol molecules should start to "feel" the presence of other cholesterol molecules. At low enough temperature, one would expect the cholesterol to be maximally separated from each other. Our results show that the simple model we have considered is able to reproduce the basic features of the superlattice model. At low temperatures, the lipid molecules are closely packed in the form of a triangular lattice and the cholesterol molecules are distributed in a regular fashion in the form of a triangular lattice of their own with a larger lattice spacing. As a measure of the cholesterol ordering, we have studied the average number of cholesterol-cholesterol nearest neighbors per cholesterol molecule. We have defined nearest neighbor as an interparticle separation of less than $1.3a$. Figure 3 shows the results from our simulations. At high temperatures, there is on average one cholesterol-cholesterol neighbor per cholesterol molecule, consistent with a disordered distribution. As the temperature is lowered, the number goes to zero continuously, signaling the setting in of cholesterol ordering. This is seen to be valid for all values of d_0 considered. We should note here that the number of nearest neighbor cholesterol molecules is not a true order parameter and Fig. 3 may or may not represent a true phase transition. This is a measure of short range order and reflects the local ordering of the cholesterol molecules.

Further information about the nature of cholesterol ordering can be obtained from the real space density correlations. Two different real space correlations have been considered—between any two molecules, and between only the choles-

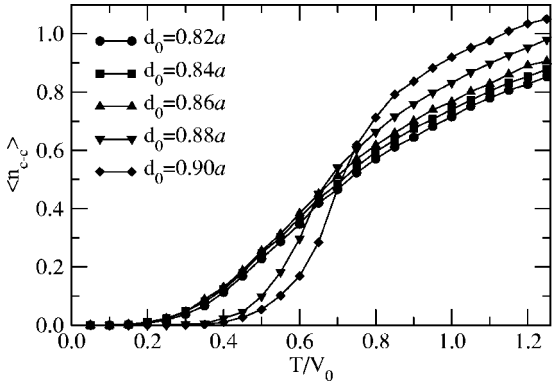

FIG. 3. The average number of cholesterol-cholesterol neighbors per cholesterol molecule as a function of temperature for several different values of the hard core radius. At low temperatures, the cholesterol molecules feel the repulsion from other cholesterol molecules and are maximally separated.

terol molecules. The results for two different values of d_0 are discussed in detail: the larger (smaller) value of d_0 corresponds to an ordered (fluid) phase in the 2D hard-disk system. Figure 4 shows the results for $d_0=0.88a$. For temperatures greater than 0.5, the real space correlations resemble that of a fluid. As the temperature is lowered below $0.5V_0$, the correlations reflect a change to a more ordered state. This is seen in both the correlations. For the cholesterol-cholesterol correlation, there is a marked shift of the first maximum from $r\approx a$ to $r\approx 1.7a$, which corresponds to the second nearest neighbor distance on a triangular lattice. This reflects the fact that the cholesterol molecules are maximally separated from one another and are arranged on the surface of the membrane in the form of a superlattice. The correlation between all species also show the signatures of a

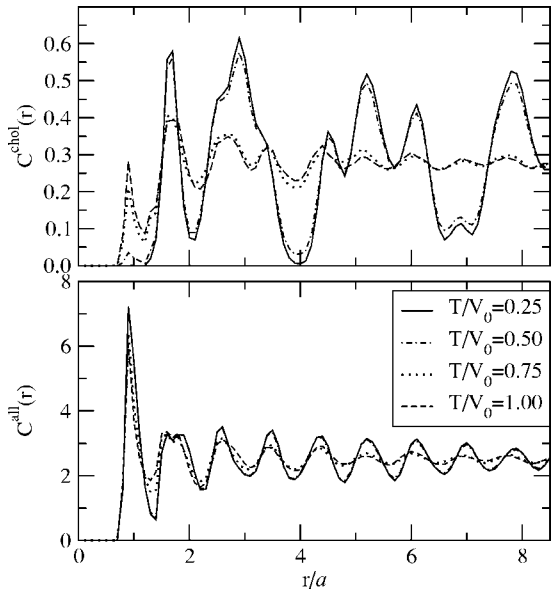

FIG. 4. The real space density correlation at different temperatures for $d_0=0.88a$. The upper panel shows the correlation between only the cholesterol molecules. The correlation between all the molecules is shown in the lower panel.

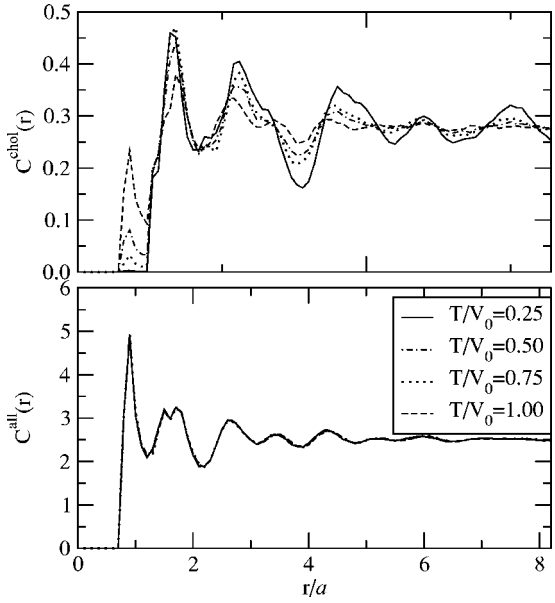

FIG. 5. The real space density correlation at different temperatures for $d_0=0.86a$. The upper panel shows the correlation between only the cholesterol molecules. The correlation between all the molecules is shown in the lower panel.

triangular-lattice-like distribution, which is consistent with close packing in two dimensions.

The behavior of the correlation functions is quite different for $d_0=0.86a$ (see Fig. 5). The cholesterol-cholesterol correlation gradually develops signatures of weak ordering, whereas the overall membrane remains fluid down to the lowest temperature explored. Locally, each cholesterol is separated from other cholesterol molecules—this is reflected in the peak of the correlation function shifting to $r\approx 1.7a$.

Figure 6 shows the snapshots at low temperatures for two systems with the two different values of d_0. For $d_0=0.88a$, the cholesterol molecules are seen to be arranged in an al-

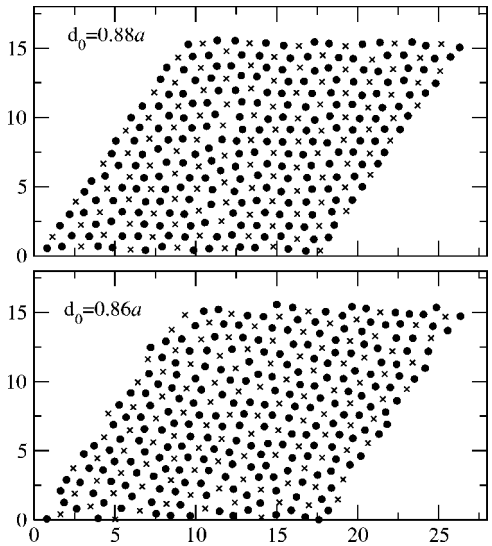

FIG. 6. Snapshots at low temperature ($T=0.10V_0$) for two systems with $d_0=0.88a$ and $0.86a$. The $\times$'s represent the cholesterol molecules and the circles denote the positions of the lipids.

most perfect superlattice. For $d_0 = 0.86a$, the system is largely fluid in nature, even though the cholesterol molecules have developed signs of (weak) ordering.

Taken together, these studies clearly show that lipid-cholesterol systems can develop lateral organization in the planes that can be called a superlattice. The development of such a pattern is primarily a local issue, being driven by the tendency of cholesterols to stay away from each other. This can happen gradually as the temperature is lowered, without the build up of long range correlations or in a more cooperative manner, where it can couple to the hexatic or bond-orientational order. We hope to develop more quantitative studies of such a coupling in the future.

IV. CONCLUSIONS

In this section we summarize our main results and suggest future research directions. The model we have proposed incorporates two distinct interactions for the cholesterol. The cholesterol molecules respond to a hard-sphere environment locally, and to a long-range repulsive interaction with each other. Thus the model sets up a competition between the ordering influence on the larger scale with a randomizing one at the local scale. The relevant parameter for the hard sphere model is the packing density. At large values of the packing density, the hard sphere model is in an ordered phase with long range bond orientational order, whereas it is in a fluid phase at low densities. The more interesting regime is at the intermediate values of the density, where the formation of the ordered cholesterol phase is sensitive to the disorder.

We have shown, via an off-lattice simulation, that even in the absence of an underlying lattice, a two-component planar system can develop lateral organization. In particular, by considering additional repulsion between one of the components, we have shown that at low temperatures they organize themselves so as to stay away from each other, resulting in a superlattice-type pattern. These results lend support to the primarily geometrical ideas of superlattice formation and magic-fraction stability in lipid cholesterol assemblies. They reinforce the view that it is not necessary to form large cholesterol-lipid condensation complexes to obtain the "magic concentrations." It may be argued that we have considered only one special cholesterol concentration that supports a local ordering. While this concentration is indeed the most favorable, it is clear by analogy that longer-range effective repulsion will similarly cause the cholesterol molecules to stay as far apart as possible. This will lead to "perfect" superlattice structure for magic concentrations when each cholesterol molecule will be surrounded by one, two, or any other integer number of lipid layers. At other concentrations, the lateral organization will consist of "mixtures" of superlattices, corresponding to a phase separation. This will explain the sharp dips observed in the fluorescence experiments.

In the future, this work can be extended in various ways. First of all, by going to a constant pressure ensemble, we can change the density with temperature, and thus drive even a hard-core system through the hexatic phase transition as a function of temperature. We can, then, study the coupling of the superlattice order to this phase transition. Furthermore, we can extend the study to more general cholesterol concentrations and study the resulting phase diagram. We can also study the fluidity and diffusion of the lipids in different phases which relates to the question: Does cholesterol ordering have a stabilizing effect on the membrane? Finally, at the expense of significantly increasing the computational complexity, one can introduce more realistic models of cholesterol and lipid molecules to study these systems. Mapping such models to simpler ones of the type considered here may be important for studying the low temperature phase behavior.

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